



SEQUENCE LISTING

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<11> Newton, Dianne L.

The Government of the United States of America
as represented by The Secretary of the
Department of Health and Human Services

<120> Selective Toxicity of Amino-Terminal Modified RNase A
Superfamily Polypeptides

<130> 015280-371100US

<140> US 09/807,556

<141> 2001-07-30

<150> US 60/106,732

<151> 1998-11-02

<150> WO PCT/US99/25737

<151> 1999-11-01

<160> 25

<170> PatentIn Ver. 2.1

<210> 1

<211> 417

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:recombinant
eosinophil derived neurotoxin with and added 4
amino acid sequence at its amino terminus
(r(-4)EDN)

<220>

<221> CDS

<222> (1)..(417)

<400> 1

atg	tca	ctc	cat	gtc	aaa	ccg	ccg	cag	ttc	act	tgg	gct	cag	tgg	ttc	48
Met	Ser	Leu	His	Val	Lys	Pro	Pro	Gln	Phe	Thr	Trp	Ala	Gln	Trp	Phe	
1				5				10					15			

gaa	act	cag	cat	atc	aac	atg	act	tct	cag	cag	tgc	act	aac	gct	atg	96
Glu	Thr	Gln	His	Ile	Asn	Met	Thr	Ser	Gln	Gln	Cys	Thr	Asn	Ala	Met	
			20				25					30				

cag	gtt	atc	aac	aac	tac	cag	cgt	cgt	tgc	aaa	aac	cag	aac	act	ttc	144
Gln	Val	Ile	Asn	Asn	Tyr	Gln	Arg	Arg	Cys	Lys	Asn	Gln	Asn	Thr	Phe	
			35				40					45				

ctg	ctg	act	act	ttc	gct	aac	gtt	gtt	aac	gtt	tgc	ggg	aac	ccg	aac	192
Leu	Leu	Thr	Thr	Phe	Ala	Asn	Val	Val	Asn	Val	Cys	Gly	Asn	Pro	Asn	
			50			55				60						

atg	act	tgc	ccg	tct	aac	aaa	act	cgt	aaa	aac	tgc	cat	cat	tct	ggg	240
Met	Thr	Cys	Pro	Ser	Asn	Lys	Thr	Arg	Lys	Asn	Cys	His	His	Ser	Gly	
			65			70				75					80	

tct	cag	ggt	ccg	ctg	atc	cat	tgc	aac	ctg	act	act	ccg	tct	ccg	cag	288
Ser	Gln	Val	Pro	Leu	Ile	His	Cys	Asn	Leu	Thr	Thr	Pro	Ser	Pro	Gln	
				85					90					95		

aac	atc	tct	aac	tgc	cgt	tac	gct	cag	act	ccg	gct	aac	atg	ttc	tac	336
Asn	Ile	Ser	Asn	Cys	Arg	Tyr	Ala	Gln	Thr	Pro	Ala	Asn	Met	Phe	Tyr	
			100					105					110			

atc	ggt	gct	tgc	gac	aac	cgt	gac	cag	cgt	cgt	gac	ccg	ccg	cag	tac	384
Ile	Val	Ala	Cys	Asp	Asn	Arg	Asp	Gln	Arg	Arg	Asp	Pro	Pro	Gln	Tyr	
			115				120					125				

ccg	ggt	ggt	ccg	ggt	cat	ctg	gac	cgt	atc	atc						417
Pro	Val	Val	Pro	Val	His	Leu	Asp	Arg	Ile	Ile						
			130				135									

<210> 2

<211> 139

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:recombinant
eosinophil derived neurotoxin with and added 4
amino acid sequence at its amino terminus
(r(-4)EDN)

<400> 2

Met	Ser	Leu	His	Val	Lys	Pro	Pro	Gln	Phe	Thr	Trp	Ala	Gln	Trp	Phe	
1				5					10					15		

Glu	Thr	Gln	His	Ile	Asn	Met	Thr	Ser	Gln	Gln	Cys	Thr	Asn	Ala	Met	
			20					25					30			

Gln	Val	Ile	Asn	Asn	Tyr	Gln	Arg	Arg	Cys	Lys	Asn	Gln	Asn	Thr	Phe	
		35					40					45				

Leu	Leu	Thr	Thr	Phe	Ala	Asn	Val	Val	Asn	Val	Cys	Gly	Asn	Pro	Asn	
	50					55					60					

Met	Thr	Cys	Pro	Ser	Asn	Lys	Thr	Arg	Lys	Asn	Cys	His	His	Ser	Gly	
	65				70					75					80	

Ser	Gln	Val	Pro	Leu	Ile	His	Cys	Asn	Leu	Thr	Thr	Pro	Ser	Pro	Gln	
				85					90					95		

Asn	Ile	Ser	Asn	Cys	Arg	Tyr	Ala	Gln	Thr	Pro	Ala	Asn	Met	Phe	Tyr	
			100					105					110			

Ile	Val	Ala	Cys	Asp	Asn	Arg	Asp	Gln	Arg	Arg	Asp	Pro	Pro	Gln	Tyr	
		115					120					125				

Pro	Val	Val	Pro	Val	His	Leu	Asp	Arg	Ile	Ile						
						130			135							

<210> 3

<211> 420

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:recombinant
eosinophil derived neurotoxin with an added 5
amino acid sequence at its amino terminus
(r(-5)EDN)

<220>

<221> CDS

<222> (1)..(420)

<400> 3

atg ggt tca ctc cat gtc aaa ccg ccg cag ttc act tgg gct cag tgg	48
Met Gly Ser Leu His Val Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp	
1 5 10 15	
ttc gaa act cag cat atc aac atg act tct cag cag tgc act aac gct	96
Phe Glu Thr Gln His Ile Asn Met Thr Ser Gln Gln Cys Thr Asn Ala	
20 25 30	
atg cag gtt atc aac aac tac cag cgt cgt tgc aaa aac cag aac act	144
Met Gln Val Ile Asn Asn Tyr Gln Arg Arg Cys Lys Asn Gln Asn Thr	
35 40 45	
ttc ctg ctg act act ttc gct aac gtt gtt aac gtt tgc ggt aac ccg	192
Phe Leu Leu Thr Thr Phe Ala Asn Val Val Asn Val Cys Gly Asn Pro	
50 55 60	
aac atg act tgc ccg tct aac aaa act cgt aaa aac tgc cat cat tct	240
Asn Met Thr Cys Pro Ser Asn Lys Thr Arg Lys Asn Cys His His Ser	
65 70 75 80	
ggt tct cag gtt ccg ctg atc cat tgc aac ctg act act ccg tct ccg	288
Gly Ser Gln Val Pro Leu Ile His Cys Asn Leu Thr Thr Pro Ser Pro	
85 90 95	
cag aac atc tct aac tgc cgt tac gct cag act ccg gct aac atg ttc	336
Gln Asn Ile Ser Asn Cys Arg Tyr Ala Gln Thr Pro Ala Asn Met Phe	
100 105 110	
tac atc gtt gct tgc gac aac cgt gac cag cgt cgt gac ccg ccg cag	384
Tyr Ile Val Ala Cys Asp Asn Arg Asp Gln Arg Arg Asp Pro Pro Gln	
115 120 125	
tac ccg gtt gtt ccg gtt cat ctg gac cgt atc atc	420
Tyr Pro Val Val Pro Val His Leu Asp Arg Ile Ile	
130 135 140	

<210> 4

<211> 140

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:recombinant
eosinophil derived neurotoxin with an added 5
amino acid sequence at its amino terminus
(r(-5)EDN)

<400> 4

Met Gly Ser Leu His Val Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp	
1 5 10 15	

Phe	Glu	Thr	Gln	His	Ile	Asn	Met	Thr	Ser	Gln	Gln	Cys	Thr	Asn	Ala	
			20					25					30			
Met	Gln	Val	Ile	Asn	Asn	Tyr	Gln	Arg	Arg	Cys	Lys	Asn	Gln	Asn	Thr	
		35					40					45				
Phe	Leu	Leu	Thr	Thr	Phe	Ala	Asn	Val	Val	Asn	Val	Cys	Gly	Asn	Pro	
	50					55					60					
Asn	Met	Thr	Cys	Pro	Ser	Asn	Lys	Thr	Arg	Lys	Asn	Cys	His	His	Ser	
	65				70					75					80	
Gly	Ser	Gln	Val	Pro	Leu	Ile	His	Cys	Asn	Leu	Thr	Thr	Pro	Ser	Pro	
				85					90					95		
Gln	Asn	Ile	Ser	Asn	Cys	Arg	Tyr	Ala	Gln	Thr	Pro	Ala	Asn	Met	Phe	
			100					105					110			
Tyr	Ile	Val	Ala	Cys	Asp	Asn	Arg	Asp	Gln	Arg	Arg	Asp	Pro	Pro	Gln	
		115					120					125				
Tyr	Pro	Val	Val	Pro	Val	His	Leu	Asp	Arg	Ile	Ile					
	130					135					140					

<210> 5

<211> 402

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:recombinant
eosinophil derived neurotoxin (rEDN)

<220>

<221> CDS

<222> (1)..(402)

<400> 5

aaa	ccg	ccg	cag	ttc	act	tgg	gct	cag	tgg	ttc	gaa	act	cag	cat	atc	48
Lys	Pro	Pro	Gln	Phe	Thr	Trp	Ala	Gln	Trp	Phe	Glu	Thr	Gln	His	Ile	
1				5				10					15			
aac	atg	act	tct	cag	cag	tgc	act	aac	gct	atg	cag	gtt	atc	aac	aac	96
Asn	Met	Thr	Ser	Gln	Gln	Cys	Thr	Asn	Ala	Met	Gln	Val	Ile	Asn	Asn	
			20					25					30			
tac	cag	cgt	cgt	tgc	aaa	aac	cag	aac	act	ttc	ctg	ctg	act	act	ttc	144
Tyr	Gln	Arg	Arg	Cys	Lys	Asn	Gln	Asn	Thr	Phe	Leu	Leu	Thr	Thr	Phe	
		35					40					45				
gct	aac	gtt	gtt	aac	gtt	tgc	ggg	aac	ccg	aac	atg	act	tgc	ccg	tct	192
Ala	Asn	Val	Val	Asn	Val	Cys	Gly	Asn	Pro	Asn	Met	Thr	Cys	Pro	Ser	
	50					55					60					
aac	aaa	act	cgt	aaa	aac	tgc	cat	cat	tct	ggg	tct	cag	gtt	ccg	ctg	240
Asn	Lys	Thr	Arg	Lys	Asn	Cys	His	His	Ser	Gly	Ser	Gln	Val	Pro	Leu	
	65				70					75					80	
atc	cat	tgc	aac	ctg	act	act	ccg	tct	ccg	cag	aac	atc	tct	aac	tgc	288
Ile	His	Cys	Asn	Leu	Thr	Thr	Pro	Ser	Pro	Gln	Asn	Ile	Ser	Asn	Cys	
				85					90						95	

cgt tac gct cag act ccg gct aac atg ttc tac atc gtt gct tgc gac 336
 Arg Tyr Ala Gln Thr Pro Ala Asn Met Phe Tyr Ile Val Ala Cys Asp
 100 105 110

aac cgt gac cag cgt cgt gac ccg ccg cag tac ccg gtt gtt ccg gtt 384
 Asn Arg Asp Gln Arg Arg Asp Pro Pro Gln Tyr Pro Val Val Pro Val
 115 120 125

cat ctg gac cgt atc atc 402
 His Leu Asp Arg Ile Ile
 130

<210> 6
 <211> 134
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:recombinant
 eosinophil derived neurotoxin (rEDN)

<400> 6
 Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe Glu Thr Gln His Ile
 1 5 10 15

Asn Met Thr Ser Gln Gln Cys Thr Asn Ala Met Gln Val Ile Asn Asn
 20 25 30

Tyr Gln Arg Arg Cys Lys Asn Gln Asn Thr Phe Leu Leu Thr Thr Phe
 35 40 45

Ala Asn Val Val Asn Val Cys Gly Asn Pro Asn Met Thr Cys Pro Ser
 50 55 60

Asn Lys Thr Arg Lys Asn Cys His His Ser Gly Ser Gln Val Pro Leu
 65 70 75 80

Ile His Cys Asn Leu Thr Thr Pro Ser Pro Gln Asn Ile Ser Asn Cys
 85 90 95

Arg Tyr Ala Gln Thr Pro Ala Asn Met Phe Tyr Ile Val Ala Cys Asp
 100 105 110

Asn Arg Asp Gln Arg Arg Asp Pro Pro Gln Tyr Pro Val Val Pro Val
 115 120 125

His Leu Asp Arg Ile Ile
 130

<210> 7
 <211> 83
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:5' primer
 oligonucleotide

<400> 7
 atatatctag aaataatttt gtttaacttt aagaaggaga tatacatatg tcaactccatg 60
 tcaaaccgcc gcagttcact tgg 83

<210> 8
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:3' primer
oligonucleotide

<400> 8
gttcacatctgg accgtatcat ctagtaggga tccgcgcg

38

<210> 9
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:N-terminus of
modified RNase A superfamily polypeptide

<220>
<221> MOD_RES
<222> (1)
<223> Met at position 1 may be present or absent

<220>
<221> MOD_RES
<222> (2)
<223> Gly at position 2 may be present or absent

<220>
<221> MOD_RES
<222> (5)
<223> Xaa = any amino acid

<400> 9
Met Gly Ser Leu Xaa Val
1 5

<210> 10
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:N-terminus of
modified RNase A superfamily polypeptide

<400> 10
Met Gly Ser Leu His Val
1 5

<210> 11
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:N-terminus of
modified RNase A superfamily polypeptide

<400> 11
 Met Ser Leu His Val
 1 5

<210> 12
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:N-terminus of
 modified RNase A superfamily polypeptide

<220>
 <221> MOD_RES
 <222> (5)
 <223> Xaa = any amino acid

<400> 12
 Met Gly Ser Leu Xaa Val
 1 5

<210> 13
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:N-terminus of
 modified RNase A superfamily polypeptide

<220>
 <221> MOD_RES
 <222> (4)
 <223> Xaa = any amino acid

<400> 13
 Met Ser Leu Xaa Val
 1 5

<210> 14
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:N-terminus of
 modified RNase A superfamily polypeptide

<220>
 <221> MOD_RES
 <222> (4)
 <223> Xaa = any amino acid

<400> 14
 Gly Ser Leu Xaa Val
 1 5

<210> 15
 <211> 4
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:N-terminus of
 modified RNase A superfamily polypeptide

 <220>
 <221> MOD_RES
 <222> (3)
 <223> Xaa = any amino acid

 <400> 15
 Ser Leu Xaa Val
 1

 <210> 16
 <211> 4
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:N-terminus of
 modified RNase A superfamily polypeptide

 <400> 16
 Ser Leu His Val
 1

 <210> 17
 <211> 5
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:peptide
 sequence inserted to allow more flexibility for
 the folding of (-4)rhEDN

 <400> 17
 Gly Gly Gly Gly Ser
 1 5

 <210> 18
 <211> 111
 <212> PRT
 <213> Rana catesbeiana

 <220>
 <223> frog lectin from Rana catesbeiana

 <400> 18
 Glu Asn Trp Ala Thr Phe Gln Gln Lys His Ile Ile Asn Thr Pro Ile
 1 5 10 15
 Ile Asn Cys Asn Thr Ile Met Asp Asn Asn Ile Tyr Ile Val Gly Gly
 20 25 30

Gln Cys Lys Arg Val Asn Thr Phe Ile Ile Ser Ser Ala Thr Thr Val
 35 40 45
 Lys Ala Ile Cys Thr Gly Val Ile Asn Met Asn Val Leu Ser Thr Thr
 50 55 60
 Arg Phe Gln Leu Asn Thr Cys Thr Arg Thr Ser Ile Thr Pro Arg Pro
 65 70 75 80
 Cys Pro Tyr Ser Ser Arg Thr Glu Thr Asn Tyr Ile Cys Val Lys Cys
 85 90 95
 Glu Asn Gln Tyr Pro Val His Phe Ala Gly Ile Gly Arg Cys Pro
 100 105 110

<210> 19
 <211> 104
 <212> PRT
 <213> Homo sapiens

<220>
 <223> eosinophil derived neurotoxin protein (EDN)

<400> 19
 Glu Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg Asp
 1 5 10 15
 Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys Asp
 20 25 30
 Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile Cys
 35 40 45
 Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe Tyr
 50 55 60
 Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu Lys
 65 70 75 80
 Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro Val
 85 90 95
 His Phe Val Gly Val Gly Ser Cys
 100

<210> 20
 <211> 133
 <212> PRT
 <213> Homo sapiens

<220>
 <223> eosinophil cationic protein (ECP)

<400> 20
 Arg Pro Pro Gln Phe Thr Arg Ala Gln Trp Phe Ala Ile Gln His Ile
 1 5 10 15
 Ser Leu Asn Pro Pro Arg Cys Thr Ile Ala Met Arg Ala Ile Asn Asn
 20 25 30

Tyr Arg Trp Arg Cys Lys Asn Gln Asn Thr Phe Leu Arg Thr Thr Phe
 35 40 45
 Ala Asn Val Val Asn Val Cys Gly Asn Gln Ser Ile Arg Cys Pro His
 50 55 60
 Asn Arg Thr Leu Asn Asn Cys His Arg Ser Arg Phe Arg Val Pro Leu
 65 70 75 80
 Leu His Cys Asp Leu Ile Asn Pro Gly Ala Gln Asn Ile Ser Asn Cys
 85 90 95
 Arg Tyr Ala Asp Arg Pro Gly Arg Arg Phe Tyr Val Val Ala Cys Asp
 100 105 110
 Asn Arg Asp Pro Arg Asp Ser Pro Arg Tyr Pro Val Val Pro Val His
 115 120 125
 Leu Asp Thr Thr Ile
 130

<210> 21
 <211> 125
 <212> PRT
 <213> Bos sp.

<220>
 <223> bovine angiogenin (Ang)

<400> 21
 Ala Gln Asp Asp Tyr Arg Tyr Ile His Phe Leu Thr Gln His Tyr Asp
 1 5 10 15
 Ala Lys Pro Lys Gly Arg Asn Asp Glu Tyr Cys Phe His Met Met Lys
 20 25 30
 Asn Arg Arg Leu Thr Arg Pro Cys Lys Asp Arg Asn Thr Phe Ile His
 35 40 45
 Gly Asn Lys Asn Asp Ile Lys Ala Ile Cys Glu Asp Arg Asn Gly Gln
 50 55 60
 Pro Tyr Arg Gly Asp Leu Arg Ile Ser Lys Ser Glu Phe Gln Ile Thr
 65 70 75 80
 Ile Cys Lys His Lys Gly Gly Ser Ser Arg Pro Pro Cys Arg Tyr Gly
 85 90 95
 Ala Thr Glu Asp Ser Arg Val Ile Val Val Gly Cys Glu Asn Gly Leu
 100 105 110
 Pro Val His Phe Asp Glu Ser Phe Ile Thr Pro Arg His
 115 120 125

<210> 22
 <211> 124
 <212> PRT
 <213> Bos sp.

<220>
 <223> bovine seminal RNase

<400> 22
 Lys Glu Ser Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Gly
 1 5 10 15
 Asn Ser Pro Ser Ser Ser Ser Asn Tyr Cys Asn Leu Met Met Cys Cys
 20 25 30
 Arg Lys Met Thr Gln Gly Lys Cys Lys Pro Val Asn Thr Phe Val His
 35 40 45
 Glu Ser Leu Ala Asp Val Lys Ala Val Cys Ser Gln Lys Lys Val Thr
 50 55 60
 Cys Lys Asn Gly Gln Thr Asn Cys Tyr Gln Ser Lys Ser Thr Met Arg
 65 70 75 80
 Ile Thr Asp Cys Arg Glu Thr Gly Ser Ser Lys Tyr Pro Asn Cys Ala
 85 90 95
 Tyr Lys Thr Thr Gln Val Glu Lys His Ile Ile Val Ala Cys Gly Gly
 100 105 110
 Lys Pro Ser Val Pro Val His Phe Asp Ala Ser Val
 115 120

<210> 23
 <211> 124
 <212> PRT
 <213> Bos sp.

<220>
 <223> bovine pancreatic RNase A

<400> 23
 Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Ser
 1 5 10 15
 Thr Ser Ala Ala Ser Ser Ser Asn Tyr Cys Asn Gln Met Met Lys Ser
 20 25 30
 Arg Asn Leu Thr Lys Asp Arg Cys Lys Pro Val Asn Thr Phe Val His
 35 40 45
 Glu Ser Leu Ala Asp Val Gln Ala Val Cys Ser Gln Lys Asn Val Ala
 50 55 60
 Cys Lys Asn Gly Gln Thr Asn Cys Tyr Gln Ser Tyr Ser Thr Met Ser
 65 70 75 80
 Ile Thr Asp Cys Arg Glu Thr Gly Ser Ser Lys Tyr Pro Asn Cys Ala
 85 90 95
 Tyr Lys Thr Thr Gln Ala Asn Lys His Ile Ile Val Ala Cys Glu Gly
 100 105 110
 Asn Pro Val Val Pro Val His Phe Asp Ala Ser Val
 115 120

<210> 24
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:N-terminus of
rEDN

<400> 24
Met Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe
1 5 10

<210> 25
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:N-terminus of
(-4)rEDN

<400> 25
Met Ser Leu His Val Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe
1 5 10 15